MONA AND MONITO ISLAND-PUERTO RICO

HAWKSBILL TURTLE RESEARCH PROJECT

RESEARCH REPORT FOR 2003

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Research Report for 2003

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MONA AND MONITO ISLAND
HAWKSBILL TURTLE RESEARCH PROJECT

INTRODUCTION

Since 1992, the Mona and Monito Island Hawksbill Research Project has surveyed and studied the ecology and population dynamics of hawksbill turtles in Mona Island Natural Reserve. The main objective of this project is to evaluate trends in the size of the hawksbill aggregations at the feeding and nesting grounds. Also, we gather important biological data on the life history and ecology that can be applied in population models and sustainable use evaluations. The high numbers hawksbill turtles at all size classes found in Mona and Monito Islands together with our sampling methods, is providing information regarding sex ratios, nest production, population densities, somatic growth rates, migration patterns and molecular studies. During the 2003 field season we concentrated our efforts in continuing the in-water surveys and nests counts. Also, we attached satellite transmitters to male and female hawksbills and started a genetic study on the origin and reproductive strategies of breeding male hawksbills that aggregates on Mona Island.

The following report documents a summary of the activities and research findings during the 2003 field season. Also, we include the preliminary results of the reproductive behavior study on male hawksbills and a comprehensive report on the nesting surveys at Mona Island. In addition, we are attaching an appendix with the list of turtles captured and tagged during this season and another appendix with the abstracts of our most recent symposium presentations and a copy of our newest peer-review publication.

IN-WATER AND NESTING SURVEYS

METHODS AND RESULTS

Study area

Mona and Monito Islands are located in the middle of the Mona Passage between the islands of Hispaniola and Puerto Rico (18°05'N, 67°54'W). These islands are uninhabited natural reserves managed by the Puerto Rico Departamento de Recursos Naturales y Ambientales. Sea turtles have been formally protected there since the introduction of the Endangered Species Act of 1973. In 1982 the U.S. Fish and Wildlife Service declared the beaches of Mona Island Critical Nesting Habitat for hawksbill turtles. On September 2, 1998, the "waters extending seaward 3 nm from the mean high water line" of Mona and Monito Islands were declared Critical Habitat for hawksbill turtles by the US National Marine Fisheries Service.

In-water turtle capture

During 2003, in-water survey data was collected from August 8 to October 8 with additional short visits to Mona for censuses and other purposes throughout the year. Our methods continue to be the same as previously described in other reports.

A total of 71.9 hours of in-water survey were registered during 2003 field season. The catch per unit effort (CPUE) involved with the sighting and capturing of turtles in our main study sites (the Carabinero-Mujeres coral reefs and the cliff walls of El Norte and Monito Island, see Figure 1) was determined by measuring the time spent for each survey session, with one survey hour defined as a unit of effort. The overall calculated CPUE for our main study sites during the 2003 field season was 10.26 hawksbill per hour (captures + sightings) with on average 3.8 persons in the water. This overall CPUE indicates a significant increase in the number of hawksbill caught and sighted from the year 1999 to 2000 and continues its high numbers until present (see Figure 2). Table 1 gives a breakdown of CPUE's by survey sites for 2003. The cliff wall habitats of Monito continues to have an exceptionally high density of hawksbill turtles, with on average over 19 turtles sighted and/or captured per hour.
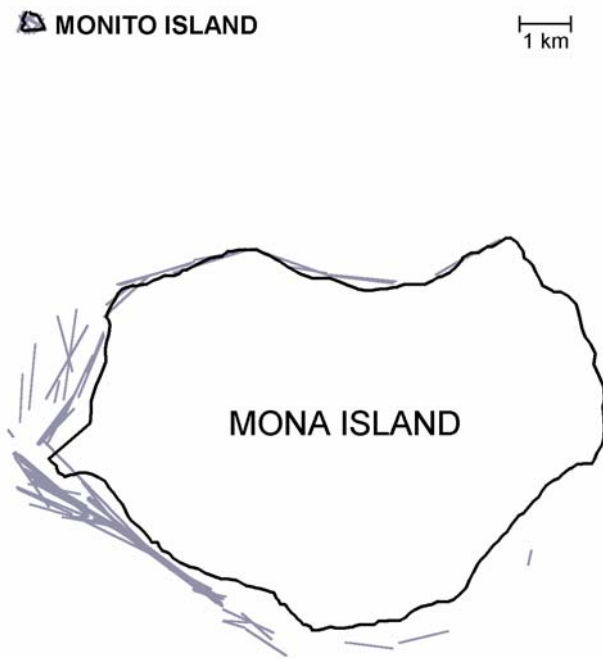
Figure 1 a.
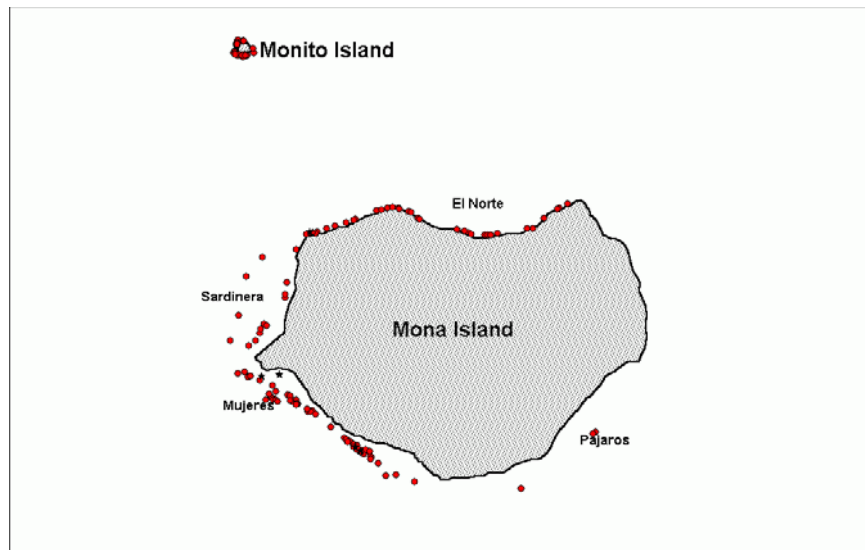
Figure 1b.

Figure 1. Maps of Mona Island with the approximate location of turtle survey transects (a) and locations where hawksbills and greens were captured (b): 2003.

Figure 3 depicts the aggregation trends for our main study sites throughout the years. These trends varied according to site when pooled data from other years. Not all the turtles captured were recorded in the CPUE because of the different methodology applied.

Table 1. Catch per unit of effort (CPUE) with standard deviation and error for each of the survey sites covered in 2003.

Survey sites	Hours	Captures + sightings per hour	SD	SE
Mona: Carabinero-Mujeres (reef)	43.4	5.09	3.03	0.22
Mona: Sardinera-Carmelitas (reef)	8.7	1.91	2.20	0.41
Mona: El Norte (cliff wall)	11.3	6.35	3.50	0.48
Mona: other areas (reef)	5.20	3.70	2.47	0.56
Monito (cliff wall)	7.6	19.33	5.92	0.81
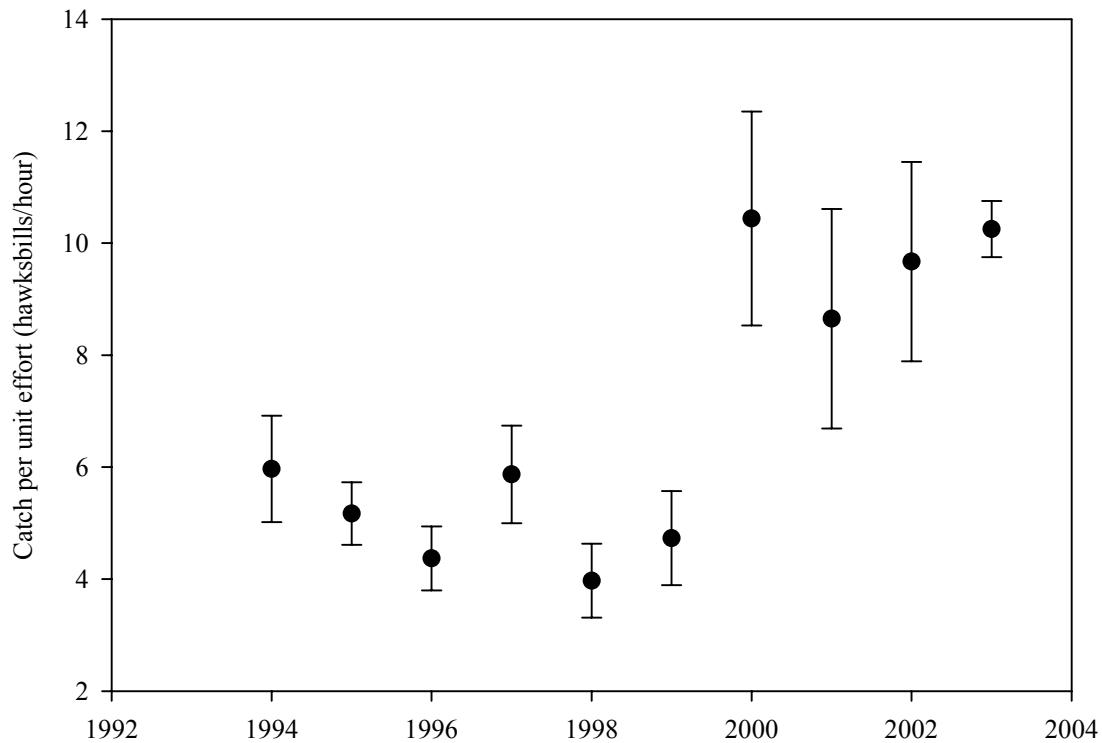
Figure 2. Overall catch per unit effort of hawksbills caught at Mona Island (1994-2003). Y-error bars denote plus and minus one standard error.
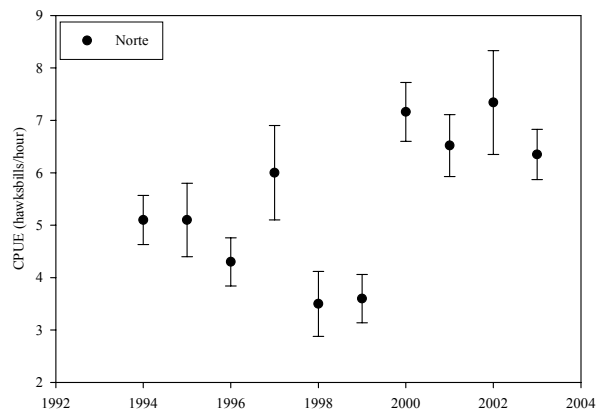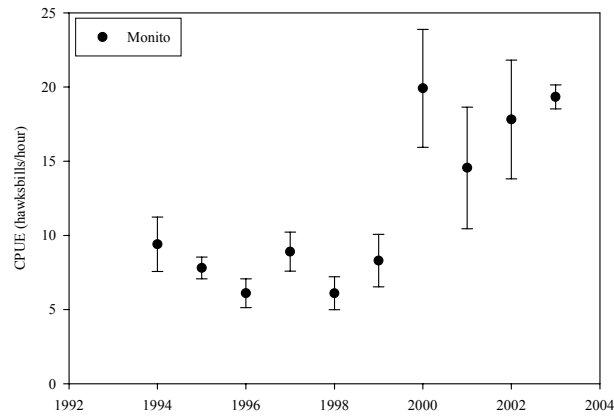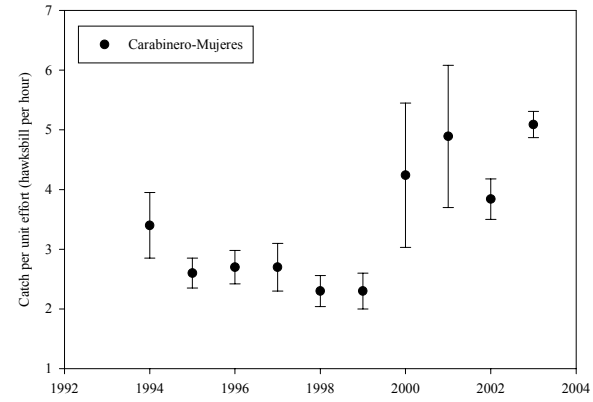
Figure 3. Catch per unit effort for our main study sites. Y error bars denote plus and and minus one standard error: 1994-2003.

During our two month-long presence on Mona during 2003, we captured a total of 156 hawksbill including 5 green turtles. This captures turtles were caught primarily along the northwest half of Mona Island and around all of Monito Island (see Figure 1). Hawksbill turtles ranged in size from 20.7 to 89.4 cm notch-tip straight carapace length (N-T SCL), green turtles ranged from 27.1.to 56.2 cm N-T SCL. Figure 4 illustrates the size distribution for hawksbills caught during this season. Notice animals from all size classes were represented. Seventy-seven hawksbills were captured bearing tags applied in previous years (see Figure 5). Untagged hawksbill turtles measuring less than 30 cm SCL size class with dark plastron, goose-neck barnacles attached and other characteristics assigned were considered new arrivals to the study area and in 2003 we found 26 such recruits. This season's numbers of recruits were lower than other years (2000: 36; 2001: 34; 2002: 31).
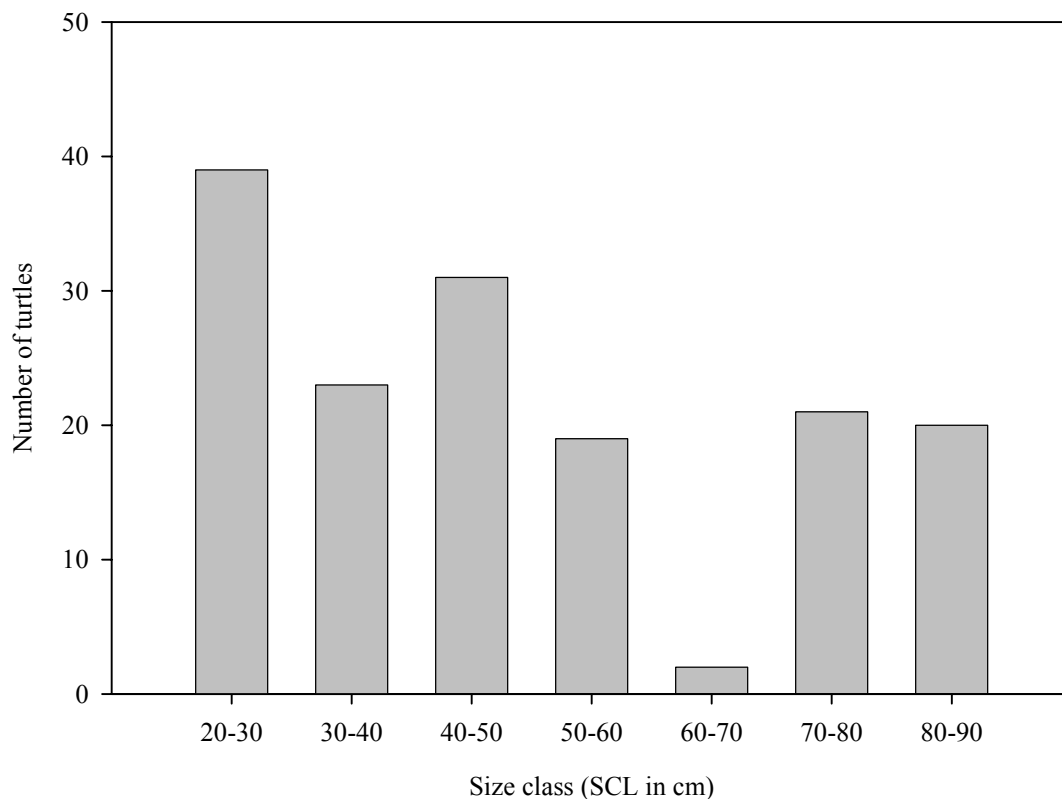
Figure 4. Size class distribution of hawksbill turltes caught at Mona Island, 2003.
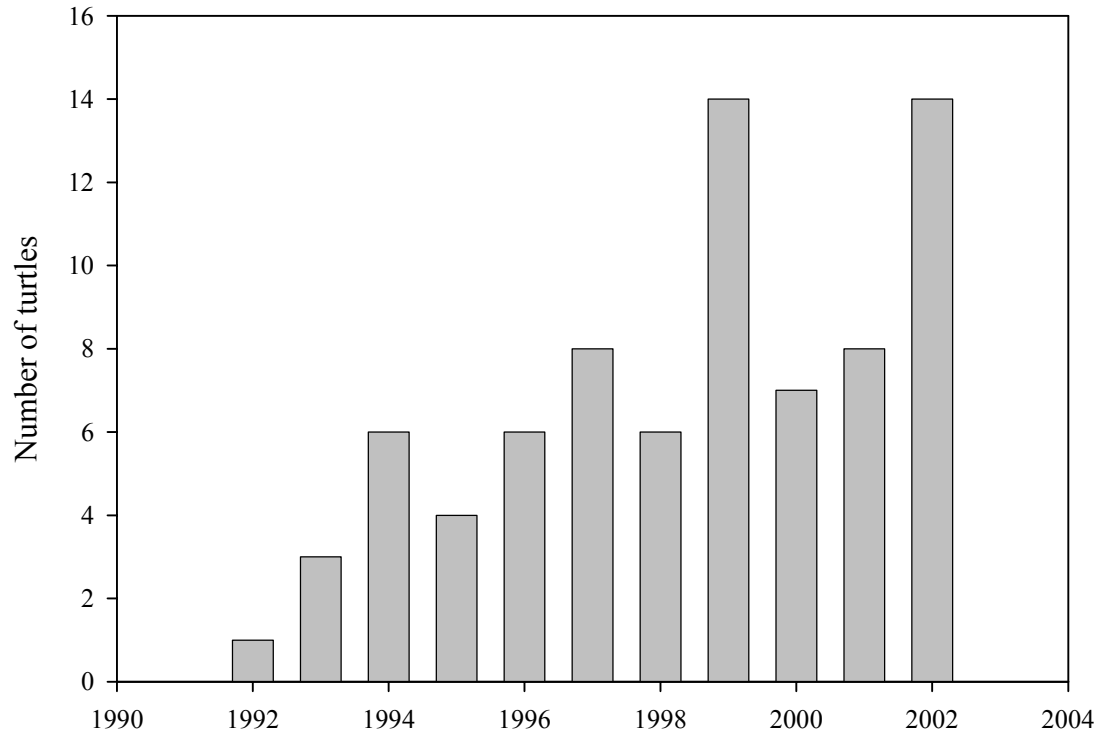
Figure 5. Distribution of hawksbill bearing tags from previous years
captured during the 2003 season, Mona Island.

Breeding male hawksbills study

Few studies have been conducted on adult male sea turtles, mainly because they spend most of their time in the ocean, different from the breeding females that have to emerge from the sea to laid eggs on the beach. Recent in-water research at Mona Island focusing on juveniles in the foraging habitats has serendipitously demonstrated the feasibility of accessing adult males for study. Therefore, this past season we initiated a study to assess the reproductive behavior of the breeding male hawksbills on Mona Island. Particularly, we are conducting research on population dynamics, determination of the migration patterns and genetic profile.

One-hundred and thirty adult male hawksbills have been captured since 1992. During 2003 field season we captured a record number of 38 individuals, due to our increased capture effort. A total of 22 recaptures from other years have been documented (see Figure 6). Most of the breeding males hawksbills have a re-migration frequency of Mona Island's breeding sites of almost every year (see Figure 7).
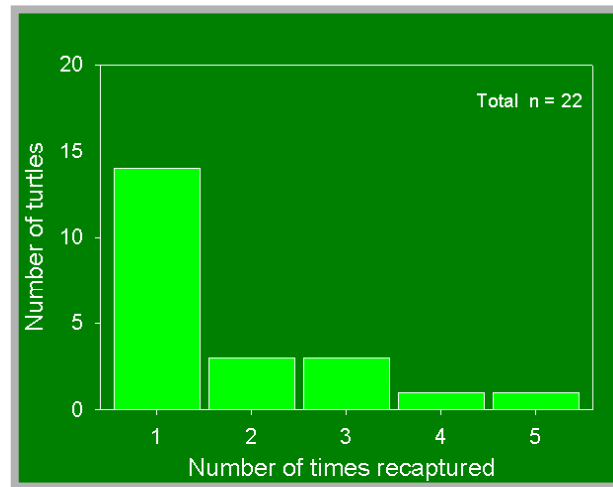
Figure 6 . Number of male hawksbill turtles and the number of times recaptured
        at Mona Island: 1992-2003.
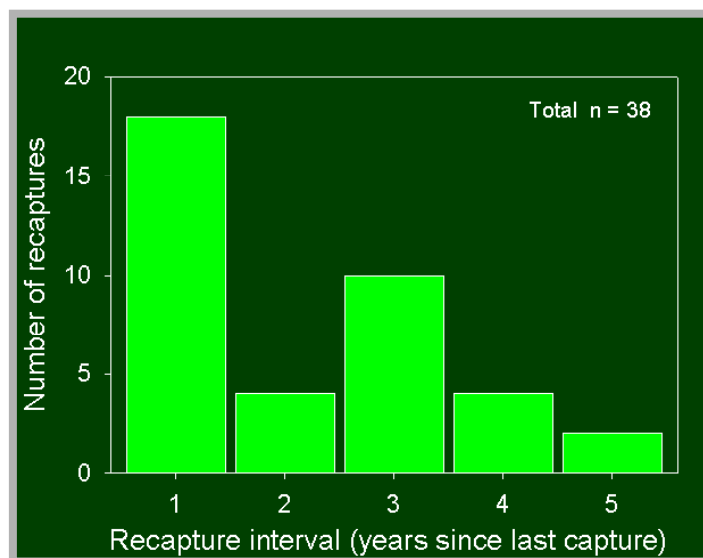
Figure 7 . The remigration frequency of adult male hawksbills in and out of
        Mona Island breeding sites as determined by capture and recapture records.

Previous tagging and telemetry studies in hawksbill turtles have focused on females and information on male migration patterns is limited. On Mona, nesting female hawksbills migrate 290-1600 km to their normal foraging grounds throughout the Caribbean, returning every two years to breed (Van Dam and Diez, unpublished data). This season we attached a satellite transmitter to one female, which moved a shorter distance than pasts female hawksbills (290 km), see Figure 8 for last location. It was assumed that males were also migratory, but preliminary tracking and tagging data suggested that males are year round residents or migrate shorter distances (see Table 2). A subset sample of five male hawksbills were fitted with satellite transmitters (see past reports for methods; 4 animals from 2002 and 1 from 2003). The data presented in figure 9 (a,b,c,d,e) illustrates the 5 male hawksbills migratory trajectories.

Table 2. Adult hawksbills straight line migration distances from Mona Island in km.

Female Distance	Year	Male Distance	Year
290	2003	<1	2002
550	1998	10	2002
570	1998	65	2002
1500	1998	150	2002
1670	1998	390	2003

Figure 8. Last location of Mona Island' post-breeding female hawksbill. March 2004.

Figure 9a.

Figure 9b.

Arrived 20-Nov-02

Deployed 13-Sep-02
Departed 12-Nov-02

Arrived 10-Oct-03
Retrieved 23-Oct-03

MONA & MONITO

150 km

Departed 27-Sep-03

Arrived 29-Nov-02

Deployed 13-Sep-02
Departed 23-Nov-02

Arrived 25-Jul-03
Retrieved 26-Aug-03

MONA & MONITO

65 km

Departed 20-Jul-03
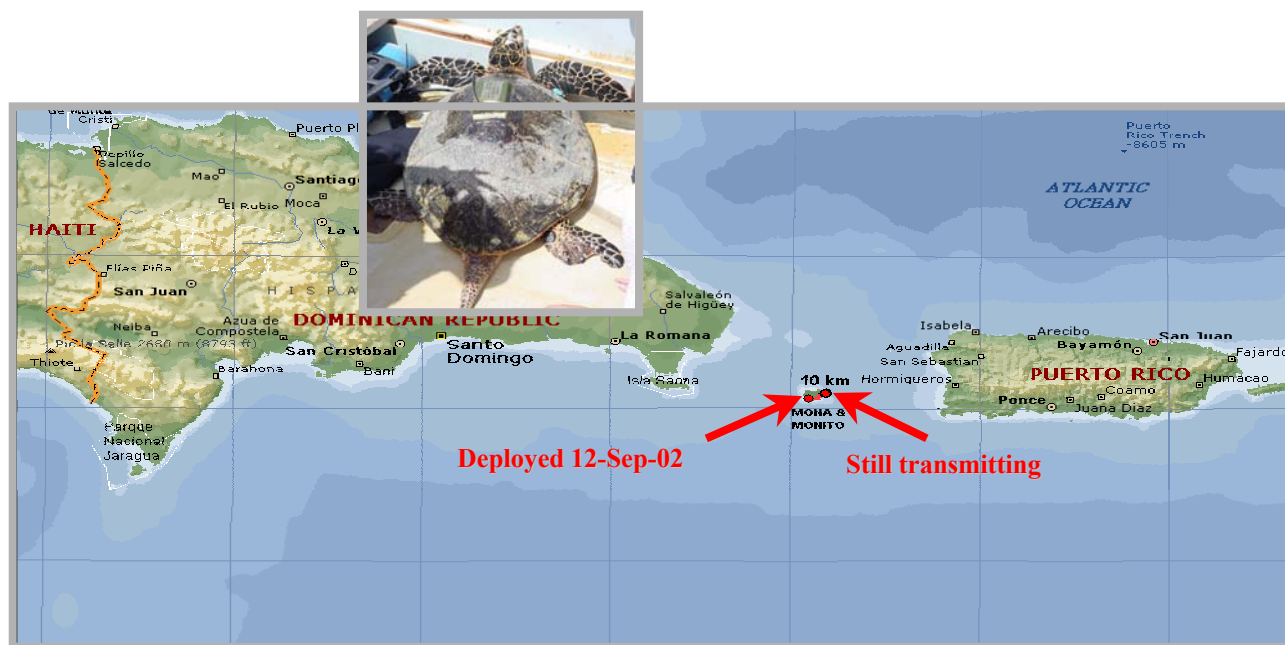
Figure 9e

Figure 9 (a,b,c,d,e). Maps with trajectories of breeding male hawksbills fitted with
satellite transmitters from Mona Island's breeding sites.

Molecular studies

Region wide genetic studies of nesting females indicates that natal homing mechanism
predominates and that nesting populations should be considered as separate stocks (Bass,
1999). Nothing is currently known about the geographic origin of males contributing to
any population of hawksbill turtles. Therefore, this season we conducted a study to
determine the nesting ground origin of adult male hawksbills from Mona Island using
molecular markers. Thirty-eight (38) tissue samples of male hawksbills were collected
for mitochondria DNA characterization to compare the results with the available maternal

genetic profiles of other hawksbill breeding populations within the Caribbean Region (Bass et al, 1996). Preliminary data indicates that male hawksbills aggregation have a higher haplotype diversity than females.

Also, we initiated a multiple paternity study to identify the maternal and paternal influences on population genetic structure for the hawksbills hatched at Mona Island. We collected tissue samples of 30-60 hatchlings from 70 different nests to conduct microsatellite analyses for a multiple paternity assessment with biparentally inherited nuclear DNA. Protocols and data analysis are still under optimization.

<u>Nesting Surveys</u>

Nesting beach surveys and hatchling success are the most common and an easiest method used among researchers to determine the trends and status of sea turtle populations. These assessments are necessary to evaluate the effects of recovery and conservation activities which are being implemented at all life history stages. Also, to understand the success of the reproductive effort of sea turtles (hatchling success), it is necessary to determine the number of hatchlings being produced. The goal of this study was to assess any trends in the nesting population of hawksbills at Mona Island.

Mona Island's 7.2 km of sandy beaches are located along the southern half of the island, with 23 named beaches. Beaches lengths vary from less than 20 meters at the small U-shaped beaches to over1 km at Mujeres beach (see map in figure 10.).

Figure 10. Map of Mona Island with names and location of nesting beaches.

Personnel conducting the nesting surveys were trained to identify nesting activities at the beginning of each season. Beaches were generally surveyed twice a week during the peak of the nesting season (July-December). All crawls were marked or "erased" to avoid duplicated counts. The crawls were classified as "false crawls" (non-nesting emergences) or nest (successful nesting). Digging for verification of nests was done only at the beginning of the season and for training purposes.

Random subsets of approximately 10% of the deposited nests were separately marked to assess nest productivity by examining their contents after hatchling emergence. Hatchling tracks and sand-surface depressions caused by cavity collapse, together with the expected emergence date, indicate a hatched nest.

Natural nest productivity P_n, defined as the number of live hatchlings produced (without human intervention) divided by the total number of eggs in the nest, is evaluated by determining its upper and lower limits. The upper limit, P_+, is established under the

assumption that all live hatchlings found in a nest would have eventually emerged on their own and made it alive to the water. Both P_+ and P- were calculated for each nest and, subsequently, the average is determined for the total of nests sampled. Means calculations are not adjusted for individual nest size. The upper and lower limit of the total number of hatchlings that reach the water's edge for the nesting season is estimated using total nest count, average nest size, and both P+ and P-.

Marine turtle nesting activity on Mona Island has been monitored from 1974, but mostly with inconsistent methods and varying survey durations. The methodological inconsistencies preclude a direct comparison of results between many of the years surveyed. The nest numbers do indicate that with a minimum estimated of 847 nest laid in 2002 and 817 nests in 2003, Mona Island's hawksbill population is the largest under U.S. jurisdiction, and the second largest in the Caribbean, after Barbados
(which has > 1, 000 nests, Horrocks, pers comm).

Nest counts were conducted during the years of 1989, 1994 thru 2003 using consistent methodology at particular months (as described above). We decided to use the same time duration or nest survey effort during a fixed period of time for establishing any increasing trends (see Figure 11).
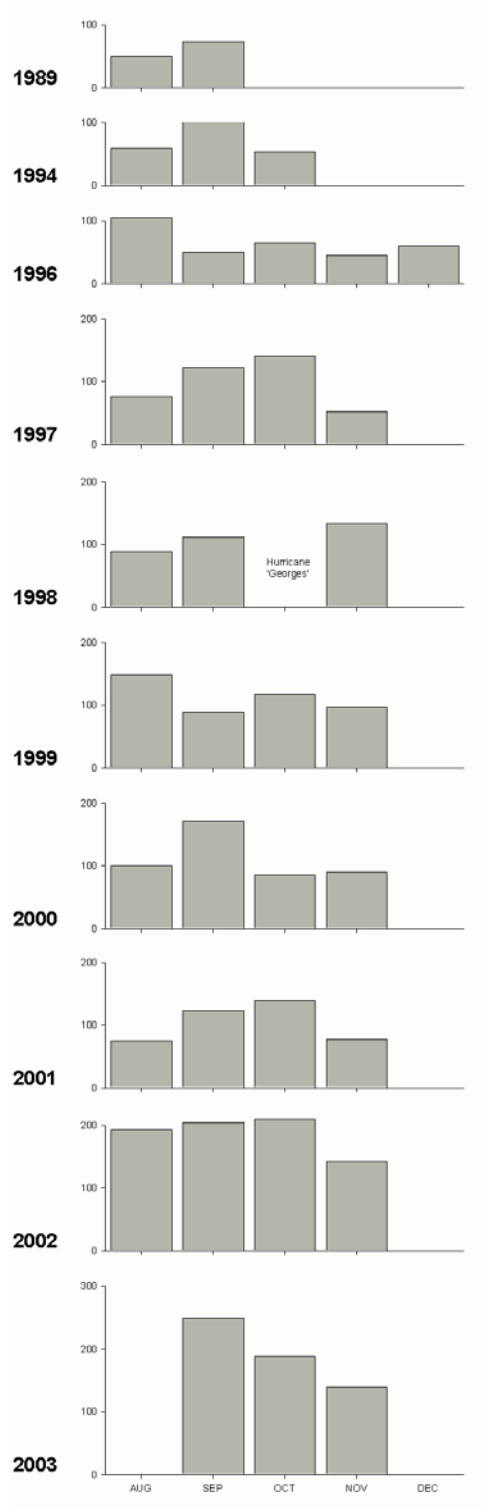
Figure 11. Hawksbill nests counts at Mona Island during 1989, 1994, 1996-2003.
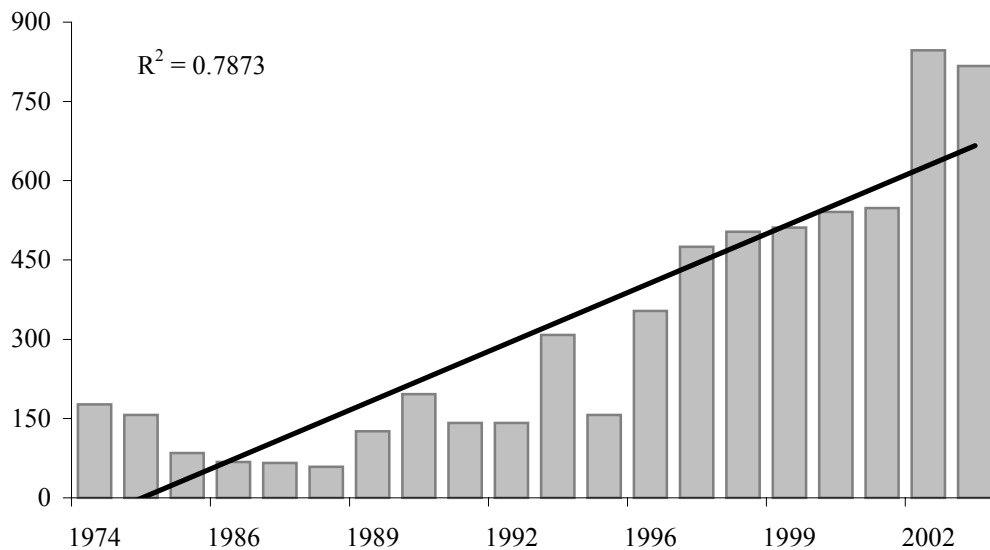
Figure 12. Number of hawksbill nests during 1974, 1984-1992; 1994-2003.

Figure 12 depicts the statistical significant upward trend in number of nests, even though the duration of survey days (effort) varied among years. An increase of 60% was reported during the last two seasons, compared with 1989 nesting season.

The number of nests also yields a large amount of hatchlings as can be seen in figure 13. In the 2003, a minimum (P-) of 78,129 hatchlings and a maximum (P+) of 96,976 were produced in Mona Island (n=154 nests).
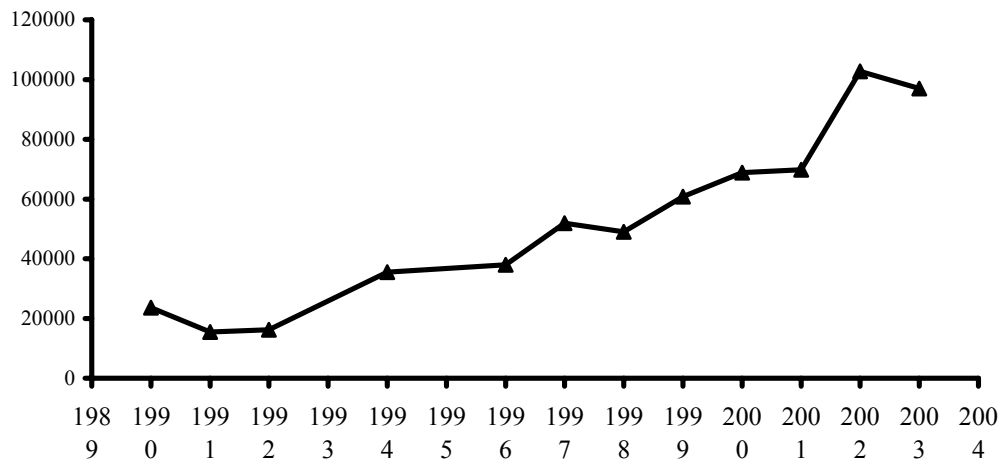
Figure 13. Number of estimated hawksbill hatchlings produced during 1990-1992; 1994; 1996-2003 at Mona Island.

We attribute the upward trend in nest numbers to the following variety of implemented conservation measures: 1)Federal and State laws have protected marine turtles in Puerto Rico since 1973; 2) the implementation of CITES to control the international trade; 3)the domestic conservation efforts from Regional States (such as Cuba and Mexico); and 4) the protection of nests from feral pigs at Mona Island since 1987. Caution should be taken into consideration when interpreting this results, since any analyses must recognize the unique status of individual nesting colonies may not reflect the general trend of the Caribbean hawksbill meta-population.

LITERATURE CITED

Bass, A. L., Good, D. A., Bjorndal, K. A., Richardson, J.I., Hillis, Z.M., Horrocks, J.A. and Bowen, B.W. 1996. Testing models of female reproductive migratory behavior and population structure in the Caribbean hawksbill turtle, *Eretmochelys imbricata*, with mtDNA sequences. Mol. Ecol. 5:321:328.

Bass, A. L. 1999. Genetic analysis to elucidate the natural history and behavior of hawksbill turtles (*Eretmochelys imbricata*) in the wider Caribbean: a review and re-analysis. Chelonian Conservation and Biology. 3(2): 189-194.

APPENDIX I. Comprehensive list of sea turtles captured during 2003 season at Mona Island, Puerto Rico.

Spe	Date capture	Turtle ID	Tag L	Tag R	PIT	N-T L
						in cm
E.i.	9-Aug-03	00-004	X7269	XBP8565		49.7
E.i.	9-Aug-03	03-002	RRA352	RRA351	062014317	33.9
E.i.	9-Aug-03	03-003	BP9251	RRA353		35.3
E.i.	9-Aug-03	94-028	SSN220	SSN221	7F7D4D0332	50.6
E.i.	10-Aug-03	03-005	RRA355	BP9248		34.7
E.i.	10-Aug-03	03-006	RRA354	BP9253		85.1
E.i.	11-Aug-03	96-077	SSN201	BP9250		55.0
E.i.	11-Aug-03	03-008	RRA356	BP9249		79.0
E.i.	11-Aug-03	01-017	XXP811	XXP810	042559797	33.3
E.i.	11-Aug-03	99-097	X7036	X7037	41054D5A4B	41.6
E.i.	11-Aug-03	99-021	X7161	BP5330		43.6
E.i.	11-Aug-03	03-012			062576639	23.3
E.i.	13-Aug-03	03-013	BP9255	RRA365		64.3
E.i.	13-Aug-03	03-014	RRA357	RRA358	050090378	27.8
E.i.	13-Aug-03	01-077	XXP845	XXP844	042585514	41.1
E.i.	13-Aug-03	03-016	RRA362	RRA361	062348296	24.6
E.i.	13-Aug-03	03-017	RRA363	RRA364	062575589	24.5
E.i.	13-Aug-03	02-035	XXP934	XXP935	050110119	34.8
E.i.	13-Aug-03	03-019	RRA360	RRA359	050101074	26.7
E.i.	13-Aug-03	96-095	BP9254	SSN213		83.8
E.i.	13-Aug-03	03-021	RRA367	BP9256		78.5
E.i.	13-Aug-03	98-076	X6943	BP1180	4150516E5C	59.4
E.i.	13-Aug-03	01-025	X7367	X7368	042593844	44.4
E.i.	13-Aug-03	99-137	X7061	X7062	410A3C1625	49.1
E.i.	13-Aug-03	03-025	RRA366	BP9262		74.1
E.i.	13-Aug-03	03-026			062538344	23.7
E.i.	13-Aug-03	01-036	XXP957	XXP819	0425740449	42.0
E.i.	13-Aug-03	99-074	X7200	X7199	410A2E1965	45.0
E.i.	14-Aug-03	02-166	RRA305	BP9246		77.3
C.m.	14-Aug-03	03-030	BP9257	RRA368		47.7
E.i.	15-Aug-03	96-097	SSN216	SSN218	N/F	51.6
E.i.	16-Aug-03	97-063	X3915	X3916	22423D375A	47.0
E.i.	16-Aug-03	03-033			062591111	23.6
E.i.	17-Aug-03	98-029	SSN249	SSN250	41504E2627	39.9
E.i.	17-Aug-03	96-022	XXP807	BP8568	4039472C32	44.0
E.i.	17-Aug-03	03-036	RRA369	RRA370	062580377	29.3
E.i.	17-Aug-03	02-003	XXP907	XXP908	049066344	30.3
E.i.	20-Aug-03	97-050	X3907	X3908	2242405B29	45.8
E.i.	20-Aug-03	98-096	XXP919	BP1190		74.6
E.i.	20-Aug-03	03-040	BP9259	RRA371		83.5
E.i.	20-Aug-03	03-041	BP9260	RRA373		84.8
E.i.	20-Aug-03	99-127	BP8547	RRA372		78.5
E.i.	21-Aug-03	95-043	SSL248	X3910		49.9

E.i.	21-Aug-03	03-044	BP9263	RRA374		87.4
E.i.	21-Aug-03	03-045	BP9264	RRA375		35.9
E.i.	22-Aug-03	02-008	BP9202	XXP912		41.0
E.i.	23-Aug-03	02-042	XXP937	BP9213		72.5
E.i.	23-Aug-03	96-009	SSM939	BP4493		89.4
E.i.	23-Aug-03	95-023	SSL240	SSL241	224217177E	48.2
E.i.	23-Aug-03	93-035	RRA377	BP1129	7F7D786B3F	53.1
E.i.	23-Aug-03	03-051	RRA376	BP9265		77.5
E.i.	24-Aug-03	93-009	1809	BP8598	7F7D394E61	52.5
E.i.	24-Aug-03	03-053			062382532	22.8
E.i.	25-Aug-03	94-122	XXP964	BP8554		80.6
E.i.	25-Aug-03	03-055	RRA379	RRA378	062270329	24.7
E.i.	25-Aug-03	99-002	BP8526	XXP938		45.9
E.i.	25-Aug-03	03-057	BP9261	RRA380		77.8
C.m.	25-Aug-03	03-058	RRA382	RRA381		27.1
E.i.	26-Aug-03	02-165	RRA304	BP9245		79.5
E.i.	27-Aug-03	03-060			062301105	20.7
E.i.	27-Aug-03	03-061	RRA386	RRA385	062599080	25.8
E.i.	27-Aug-03	94-104	SSJ986	SSJ985	1F4A4D2E1C	44.2
E.i.	27-Aug-03	94-041	X4648	BP9221	1F4A095F2F	62.2
E.i.	27-Aug-03	99-018	X7159	X7160	410A1B4E0C	34.4
E.i.	27-Aug-03	03-065	RRA383	RRA384	062869058	25.1
E.i.	27-Aug-03	03-066	RRA391	BP9266		78.5
E.i.	27-Aug-03	01-096	RRA388	RRA387	049072263	28.1
C.m.	27-Aug-03	03-068	RRA389	RRA390		29.0
E.i.	29-Aug-03	03-069	BP9267	RRA392		80.0
E.i.	29-Aug-03	03-070	BP9268	RRA393		81.5
E.i.	29-Aug-03	02-020	XXP922	XXP921	050312577	30.4
E.i.	29-Aug-03	03-072	BP9269	RRA394		78.7
E.i.	29-Aug-03	03-073	RRA396	RRA 397	062008860	26.5
E.i.	29-Aug-03	03-074	RRA395	BP9270		53.4
E.i.	29-Aug-03	99-098	X7040	X7039	41050E3E39	38.3
C.m.	30-Aug-03	03-076	RRA399	BP9275		56.2
E.i.	30-Aug-03	01-121	XXP856	XXP857	049057517	36.7
E.i.	31-Aug-03	03-078	RRA422	RRA423	061804026	26.5
E.i.	31-Aug-03	03-079			061829516	22.4
E.i.	31-Aug-03	03-080	RRA424	RRA425	050024611	25.0
E.i.	31-Aug-03	95-127	SSM980	BP9109	403730F18	48.6
E.i.	31-Aug-03	03-082	RRA398	RRA400	050262542	26.4
E.i.	31-Aug-03	03-083			050273360	23.7
E.i.	31-Aug-03	03-084	RRA421	RRA420	061893262	28.3
E.i.	31-Aug-03	98-098	X6969	X6968	410A272507	47.3
E.i.	31-Aug-03	03-086	RRA415	RRA414	062111277	26.0
E.i.	31-Aug-03	03-087	RRA417	RRA416	062028380	27.4
E.i.	31-Aug-03	02-066	RRA418	RRA419	050295073	27.2

E.i.	31-Aug-03	94-095	BP4425	SSJ974	1F482F6802	53.8
E.i.	31-Aug-03	02-062	XXP949	BP9219		33.5
E.i.	31-Aug-03	00-076	X7238	BP8596		47.0
E.i.	31-Aug-03	96-070	BP8521	SSM996		49.7
E.i.	31-Aug-03	97-119	X3959	X3958	2242165309	41.9
E.i.	2-Sep-03	97-030	SSN292	SSN291	4035736C6A	49.2
E.i.	2-Sep-03	03-095	BP9271	RRA413		78.2
E.i.	2-Sep-03	03-096	RRA412	BP9272		80.5
E.i.	2-Sep-03	00-029	BP8572	X7260		51.0
E.i.	4-Sep-03	03-098	RRA411	BP9274		79.9
E.i.	5-Sep-03	03-099	RRA410	BP9273		77.6
E.i.	5-Sep-03	03-100	RRA409	BP9287		77.6
E.i.	6-Sep-03	03-101	RRA408	BP9286		77.7
E.i.	6-Sep-03	03-102	RRA407	BP9285		81.4
E.i.	8-Sep-03	00-108	X7308	X7307	042608880	34.2
E.i.	8-Sep-03	03-104	BP9276	RRA406		51.6
E.i.	8-Sep-03	99-068	X7194	X7195	410A352201	51.5
E.i.	8-Sep-03	01-100	XXP959	XXP958	042542847	35.5
E.i.	8-Sep-03	99-030	X7167	BP9226	410A3785C	47.1
E.i.	8-Sep-03	02-106	XXP965	XXP966	050125333	32.0
E.i.	8-Sep-03	01-076	BP9112	XXP841		53.5
E.i.	8-Sep-03	03-110	BP9277	RRA405		55.2
E.i.	8-Sep-03	03-111	RRA404	RRA403	062030001	28.9
E.i.	8-Sep-03	03-112			062048020	24.4
E.i.	8-Sep-03	03-113	RRA402	RRA401	062579015	29.4
E.i.	8-Sep-03	00-034	X7257	X7254	042599816	43.5
E.i.	8-Sep-03	99-076	BP8532	X7026		57.0
E.i.	8-Sep-03	03-116	RRA426	RRA427	062594088	25.1
E.i.	8-Sep-03	03-117			062325811	24.6
E.i.	10-Sep-03	03-118			062798046	21.5
E.i.	10-Sep-03	03-119	RRA428	BP9278		86.9
E.i.	11-Sep-03	03-120	RRA429	BP9279		81.0
E.i.	12-Sep-03	03-121	BP9280	RRA430		84.8
E.i.	12-Sep-03	03-122	BP9281	RRA431		81.3
E.i.	12-Sep-03	00-015	X7265	X7266	040074278	42.5
E.i.	12-Sep-03	03-124	RRA432	RRA433	062052070	25.5
E.i.	13-Sep-03	03-125	RRA434	BP9282		78.1
E.i.	13-Sep-03	97-051	X3909	BP1140	22421F3609	55.6
E.i.	13-Sep-03	93-016	1816	BP9283	7F7D3E214E	57.3
E.i.	16-Sep-03	03-128	BP9284	RRA435		84.5
E.i.	18-Sep-03	03-129	BX1512	RRA436		83.1
E.i.	18-Sep-03	03-130	RRA438	RRA439	050101866	32.8
E.i.	18-Sep-03	03-131	BX1502	RRA437		38.7
E.i.	19-Sep-03	98-128	X6999	BP8504		80.1
E.i.	21-Sep-03	02-041	BP9212	XXP936		41.0

APPENDIX I. (continuation)

E.i.	23-Sep-03	03-134			062037577	23.3
E.i.	23-Sep-03	99-028	X7165	X7164	4105233C55	51.7
E.i.	23-Sep-03	03-136	RRA448	RRA447	061860074	32.1
E.i.	23-Sep-03	03-137	RRA442	RRA441	062029607	27.4
E.i.	23-Sep-03	02-098	RRA444	RRA443	050048006	29.4
E.i.	23-Sep-03	03-139	RRA440	BX1503		84.2
E.i.	23-Sep-03	03-140	RRA446	RRA445	050107598	24.4
C.m.	23-Sep-03	03-141	RRA450	RRA449		28.7
E.i.	24-Sep-03	02-115	RRA452	RRA451	050078296	26.0
E.i.	24-Sep-03	02-071	XXP952	XXP951	050288597	31.4
E.i.	24-Sep-03	94-026	X4631	X4632		49.7
E.i.	24-Sep-03	97-005	SSN271	SSN270		43.8
E.i.	24-Sep-03	97-003	SSN267	SSN266	40392D3933	39.8
E.i.	24-Sep-03	00-082	X7242	X7241	042538003	34.0
E.i.	24-Sep-03	97-101	X3939	X3938	22422355E12	42.7
E.i.	24-Sep-03	98-107	BP8567	X6977	410977272D	44.1
E.i.	24-Sep-03	99-100	BP8537	X7038		77.0
E.i.	25-Sep-03	99-146	X7068	BP8555		77.5
E.i.	26-Sep-03	92-045	XXP806	BP8587	7F7D32183C	54.7
E.i.	27-Sep-03	03-153	BX1504	RRA453		34.6
E.i.	28-Sep-03	95-077	BP4468	SSL271		76.6
E.i.	29-Sep-03	03-155	BX1506	RRA454		78.1
E.i.	30-Sep-03	03-156	BX1507	RRA455		80.0

APPENDIX II. Recent presentations and publications.

Vélez-Zuazo, X.; Van Dam, R.P.; Diez, C.E. *In press*. The Recovery of the Hawksbill Turtles at Mona Island, Puerto Rico: Report of Record Nesting Seasons and its Implication to the Caribbean Region. *In:* Proceedings of the 24th International Symposium on Sea Turtle Biology and Conservation. *Ed* Roderick Mast. San José, Costa Rica. 2004.

The Recovery of Hawksbill Turtles at Mona Island, Puerto Rico:
Report of Record Nesting Seasons and its Implications to the Caribbean Region

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ABSTRACT

Mona Island is considered one of the most important rookeries in the Caribbean for the critical endangered hawksbill sea turtle. A steady increase in the number of hawksbill nests in Mona Island has been reported since 1994. During 2002, we had a record number of nests (n=847) indicating a 60% increase compared to the previous year. During the current season, 2003, nesting activity appears to be similar or even higher than in 2002 (data is still being collected). Total reproductive output for Mona Island for 2002 is estimated to be over 90 000 hawksbill hatchlings. An increasing number of nesting females visiting Mona has allowed us to collect detailed data on turtle morphometrics, nesting site fidelity, clutch size, interesting periods, re-migration intervals, nest incubation durations and hatching success. The following poster will present the results of the data mentioned above, with a discussion of the factors affecting the recovery of the hawksbill nesting population at Mona Island.

Van Dam, R.P.; Diez, C.E.; Colón, L. *In press*. Close and Often: Migratory and Breeding Behavior of Male Hawksbill Turtles. *In:* Proceedings of the 24th International Symposium on Sea Turtle Biology and Conservation. *Ed* Roderick Mast. San José, Costa Rica. 2004.

Sex Ratio of an Immature Hawksbill Seaturtle Aggregation at Mona Island, Puerto Rico

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Abstract.—With nest incubation temperature determining sex in marine turtle hatchlings, sex ratios among populations and intermediate aggregations are likely to exhibit more geographic and temporal variability than for vertebrates with genotypic sex determination. In this study, we examined the sex ratio of an immature Hawksbill Seaturtle (*Eretmochelys imbricata*) aggregation at Mona Island, Puerto Rico, using serum testosterone level criteria that were validated through laparoscopy in a subset of turtles. Measured serum testosterone for female turtles ranged between 0.32 and 17 pg/ml and for males between 18.2 and 262 pg/ml. Of 120 turtles sampled, 53 individuals were classified as females and 66 as males, with one individual undetermined. The resulting F:M ratio of 0.80:1 is not significantly different from 1:1. This result contrasts with the highly female-biased sex ratios reported from surrounding Caribbean immature hawksbill aggregations, suggesting that the Mona near shore habitat recruits male turtles from a source uncommon to other aggregations.

RESUMEN. Las proporciones sexuales en tortugas marinas tienen gran importancia para la creación de planes de manejo efectivos, ya que en estas especies el sexo es determinado por la temperatura ambiental y no por diferenciación de crómosomas. Esta característica ha creado poblaciones de tortugas marinas con las proporciones sexuales sesgadas a un género en particular y no la proporción sexual de 1:1 propuesta por la teoría de alocación sexual. Varios trabajos han demostrado que estudios de proporciones sexuales en agregaciones de juveniles y sub-adultos de tortugas marinas son los más apropiados para obtener la razon sexual real de dicha agregación. Para determinar la razon sexual de una agregación de la altamente amenazada tortuga marina carey (*Eretmochelys imbricata*) en la Reserva Natural de Isla de Mona, Puerto Rico se utilizararon los niveles de testosterona como técnica para distinguir el sexo de cada individuo. De 120 individuos, el 56% fueron machos y el 44% fueron hembras. Estos resultados no reflejaron una diferencia estadísticamente significativa de la razon sexual 1:1. Sin embargo, investigaciones en otras agregaciones de la tortuga carey en el Caribe reportan sesgos sexuales hacia un género en particular. Estudios de proporciones sexuales tanto de neonatos en playas de anidación como de juveniles en áreas de alimentación de la tortuga carey en el Caribe son necesarios para ayudar a explicar la diferencia en las proporciones sexuales de las agregaciones de tortugas carey en el Caribe.

Marine turtles are subject to environmental sex determination (Bull, 1980), through sensitivity to temperature during embryonic development (Mrosovsky and Yntema, 1980). As a result, sex ratios among turtle hatchlings can differ significantly from 1:1 (Yntema and Mrosovsky, 1982; Limpus et al., 1983; Mrosovsky et al., 1984). Incubation temperatures of two other Caribbean Hawksbill Seaturtle (*Eretmochelys imbricata*) rookeries produce substantial variation in the sex ratios hatchlings (Mrosovsky et al., 1992; Wibbels et al., 1999). The way in which hatchlings of different rookeries influence the sex ratios in aggregations of immature turtles, and eventually affect sex ratios of breeding populations, remains largely unknown, because this process is governed by poorly understood factors such as migration patterns, differential survival, growth, and maturation and temporal variation in rookery hatchling sex ratios (Wibbels, 1999).

One problem in measuring sex ratio of any mature turtle population is the potential for strong sampling bias caused by behavioral differences in reproductively active turtles. Adult male hawksbills at Mona Island, for example, tend to be highly inquisitive and, therefore, more easily encountered than the shy females on the nearshore breeding grounds (pers. obs.). Any sex ratio estimates based purely on sightings or capture frequencies would be strongly biased by this behavioral difference. Previous studies lacking controls to eliminate the potential biases caused by sexual differences in turtle behavior include those on the sex ratios among harvested adult Green (*Chelonia mydas*; Ross, 1984) and Hawksbill Seaturtles (Carrillo et al., 1999). By studying immature turtles, at least some of the behaviorally related sampling biases may be avoided, and the premise of equal catchability can be tested realistically.

An important consideration of feeding ground studies of immature marine turtles is that the sex ratios encountered do not necessarily represent any one breeding population. Aggregations of immature hawksbills may be composed of individuals originating from various nesting colonies, such as in the aggregation studied at Mona Island, where animals from at least six breeding sites in the Caribbean are represented (Bass, 1999). Sex ratios of immature turtle aggregations thus can be considered the amalgamated result of the prevailing hatchling production of contributing colonies.

Reported sex ratios vary greatly between marine turtle aggregations, presumably because of differences in the thermal nest incubation conditions from where

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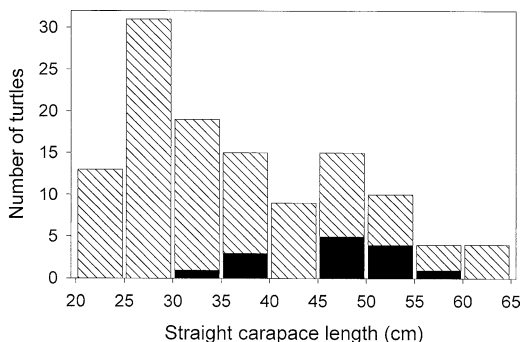
[1] Corresponding Author.

FIG. 1. Size distribution of the 120 immature Hawksbill Seaturtles studied. Black bars indicate turtles examined through laparoscopy and by blood serum testosterone assay. Hatched bars represent turtles examined by laparoscopy and through blood serum testosterone level assays. Hatched bars indicate turtles subjected only to testosterone measurement. Turtles sampled on more than one occasion are included only once, using the mean of straight carapace length measurements.

the turtles originated. Limpus et al. (1983), Wibbels et al. (1991), and León and Diez (1999) encountered bias toward either males or females on turtle feeding grounds, whereas Wibbels et al. (1993) reported an unbiased sex ratio of immature Green Seaturtles in the Hawaiian Archipelago. Limpus and Reed (1985) suggested that the mechanism of temperature-dependent sex determination could produce nonbiased sex ratios in marine turtle populations. In the current study, we validated the serum testosterone level criteria used for assigning sex and then tested the working hypothesis of an unequal sex ratio for the aggregation of immature hawksbills inhabiting the feeding grounds of Mona Island, Puerto Rico. Finally, we tested whether there were sexual differences in catchability for the group of turtles studied.

MATERIALS AND METHODS

Study Area.—The Mona Island Natural Reserve incorporates two islands, Mona and Monito. These islands are located midway in the Mona Passage between the Dominican Republic and Puerto Rico. The near-shore zones of both islands are known feeding grounds for juvenile and adult Hawksbill Seaturtles and are visited by adults nesting on Mona. Turtles from three sections of these feeding grounds are included in this study, encompassing two major habitat types: the cliff walls dominating the coast of Monito and the northern half of Mona Island; and the coral reef patches along the south-west coast of Mona.

Data Collection.—Every year from 1992 to 2000, we surveyed the study area for periods of from one to four months, generally during summer. Turtle surveys included capture of sighted Hawksbill Seaturtles by hand, supplemented by free diving or with the aid of SCUBA. All captured turtles were brought aboard a small boat for tagging and measurement, with a subset sampled for blood in the years 1993 to 1995. In addition, during a one-week period in 1993, turtles

were taken ashore for laparoscopic examination. All turtles were subsequently returned to the location of capture. Turtles with straight carapace lengths (SCL) between 20.0 and 65.0 cm were classified as immatures (van Dam and Diez, 1998).

Visual Sex Determination.—In 1993, 14 immature Hawksbill Seaturtles were examined internally by laparoscopy to determine sex by visual assessment of the gonads. Because of the size of the available laparascopy equipment, only hawksbills greater than 34 cm SCL could be examined safely. Turtles were immobilized, and an endoscope was introduced into the peritoneal cavity for visual inspection of the gonads (Wood et al., 1983). The criteria used for assigning sex to an individual followed those established by Limpus and Reed (1985) for *C. mydas*. After laparoscopy, a highly visible piece of tape was glued to the carapace of the turtles to permit behavioral observations and prevent recapture.

Blood Sampling and Processing.—From 1993 to 1995, blood was collected from 120 immature Hawksbill Seaturtles, typically 30 min after capture. Up to 10 ml blood was drawn from each animal through one of the dorsal cervical sinuses using a Vacutainer tube (without additives) and a 3.8-cm 22-ga needle. Whole blood was stored on ice until completion of each daily survey. Following centrifugation for five minutes, two 2-ml samples of blood serum per animal were extracted by pipette, labeled, and stored in a −10°C freezer. The testosterone level from one of each duplicate serum sample was measured in the laboratory of D. W. Owens using a sensitive radio-immunoassay (Owens et al., 1978; Wibbels et al., 1987).

RESULTS

The size distribution (Fig. 1) of the 120 turtles sampled for testosterone was similar to that of 276 Hawksbill Seaturtles captured during 1993–1996 (van Dam and Diez, 1998) and can be considered representative of the aggregation of immature turtles in the study area. As only larger hawksbills were selected for laparoscopy, an underrepresentation of animals < 35 cm SCL exists in this subset of turtles (Fig. 1).

Validation of Testosterone Criteria for Determining Sex.—Of the 14 hawksbills examined by laparoscopy, six individuals were classified as females and eight as males, after detecting ovarian follicles and testes, respectively. Serum testosterone concentrations of the six females ranged between 3.35 and 16.1 pg/ml, whereas testosterone of the eight males ranged between 68.4 and 262 pg/ml (Fig. 2A). Six of the turtles examined by laparoscopy were resampled for testosterone in 1994 or 1995 or both, allowing an extension of the validated hormone level range between to 3.1 and 16.1 pg/ml for females and 56.7–262 pg/ml for males (Fig. 2A',A").

During 1993–1995, an additional 32 turtles were captured and sampled on two or more occasions, yielding a range of testosterone levels for each individual. Where these individual ranges overlapped with those of the laparoscoped animals, they allowed for a further stepwise widening of the validated testosterone ranges for each of the sexes. For example, the serum testosterone level of turtle 94-067 was 14.4 pg/ml in 1994, establishing it as a female; in 1995 this individual had a level of 17.0 pg/ml, extending the
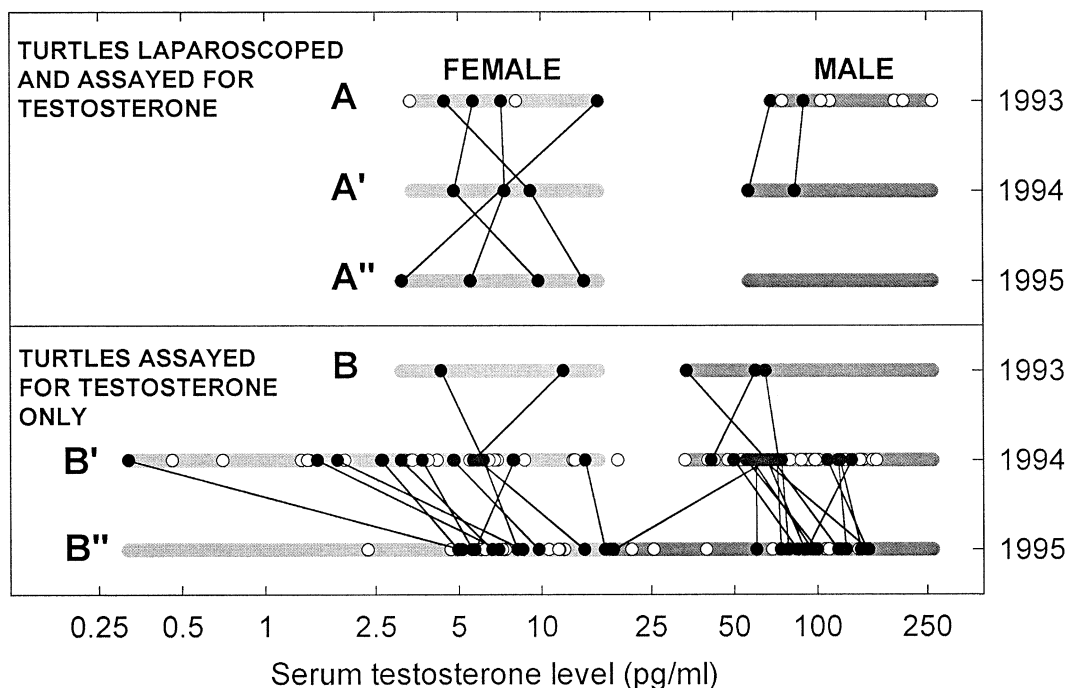
FIG. 2. Construction of testosterone level criteria for assigning sex to immature Hawksbill Seaturtles captured at Mona Island, Puerto Rico. Turtles with sex determined laparoscopic examination (A) yielded an initial range (shaded) of associated testosterone levels; extension of range through subsequently measured testosterone levels of these laparoscoped individuals (connecting lines: A, A′ and A″). Assignment of sex to turtles sampled for testosterone only (B) using range from A″, and the expansion of the female and male range limits from turtles sampled on multiple occasions (connecting lines: B, B′, B″). Hormone levels of turtles sampled only once are plotted as open circles.

previous upper testosterone limit for females to this value. Such extension by individual duplicates resulted in a serum testosterone range of between 0.32 and 17 pg/ml for females and between 18.2 and 262 pg/ml for males (Fig. 3B,B′,B″). These extensions yielded no inconsistencies or overlap in the serum testosterone ranges assigned to females and males.

Sex Ratio.—With the established serum testosterone ranges, only one hawksbill (with the intermediate level of 17.7 pg/ml) of the 120 turtles sampled remained of undeterminable sex. Exclusion of this individual from the dataset resulted in a total count of 53 females and 66 males. This F:M proportion of 0.80:1 was not significantly different from 1:1 (χ^2 = 1.42, P = 0.233).

As the lower limit of the male range of testosterone levels was large (from the 56.7 pg/ml validated by laparoscopy down to 18.2 pg/ml by comparison of individual duplicates), we tested the sensitivity of the overall resulting sex ratio through stepwise reversals of this extension. Because the lower limit for males was raised from 18.2 to 56.7 pg/ml, progressively more turtles were assigned to the undetermined sex category, yielding new calculated sex ratios (Fig. 3). With more conservative settings for the lower limit, the F:M sex ratio steadily approached a value of 1:1, (not significantly different from 1:1). Similarly, exclusion from the dataset of hawksbills smaller than 34.0 cm SCL (size of the smallest laparoscoped turtle), resulted in a sex ratio

F:M of 0.72:1 among the remaining 52 individuals. This ratio was not significantly different from 1:1 (χ^2 = 1.473, P = 0.225).

Testing Equal Catchability.—An assumption implicit in studying aggregation characteristics is an even probability of sampling the different subgroups of interest. To test for a possible difference in catchability between the sexes, we compared the number of times turtles of each sex were caught (counting any number of captures within a single year as one). From 1993 to 2000, the 53 female and 66 male turtles examined in the present study were captured on average 3.04 and 3.05 times, respectively. The most parsimonious explanation for the closeness of these values is that there was no sex-related catchability bias for this group of immature hawksbill turtles.

DISCUSSION

The even sex ratio measured for Mona Island's immature hawksbill aggregation contrasts with the strongly female-biased sex ratios reported for other aggregations off the southwest coast of the Dominican Republic (F:M = 2.71:1, N = 143, 1996–1998; León and Diez, 1999), and around Buck Island in the U.S. Virgin Islands (F:M = 4:1, N = 72, 1996–1998; Geis et al., in press). Although the current study pre-dates the others by several years, a strictly temporal explanation for the
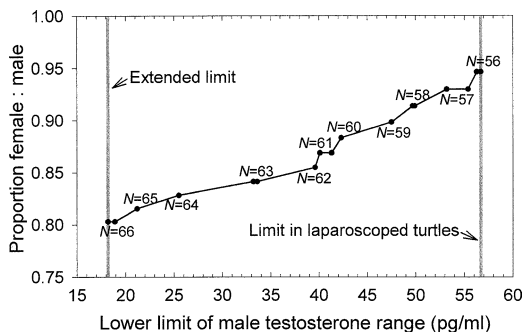
Fig. 3. Sensitivity of the calculated sex ratio to the lower limit of testosterone for designating Hawksbill Seaturtles as males. As the limit is set more conservatively (higher), turtles with hormone levels lower than the limit (but above 17 pg/ml, the firm female upper limit) are progressively assigned to the category "sex unknown," reducing the number (N) of turtles determined as males. For all points in the graph, the resulting F:M sex ratio remains statistically not different from 1:1.

observed differences is implausible, because all studies included a range of turtle sizes corresponding to animals recruited to the feeding grounds over a period of around a decade (assuming recruits initially measured < 30 cm SCL and had somatic growth rates of around 3 cm SCL/year). The effect of any hypothetical influx of highly female-biased turtles recruiting to the region's feeding grounds after 1995 is unlikely to have been detected because of their dilution within the resident pool of animals and the relatively short sampling periods.

The contrasting sex ratios are more probably attributable to dissimilar sourcing, that is, differences in the nesting rookeries contributing individuals to the feeding ground aggregations. With nest incubation temperatures determining hatchling sex in Hawksbill Seaturtles (Mrosovsky et al., 1992), the Mona aggregation appears to have received a high proportion of turtles hatched on cooler, male-generating nesting beaches. We are aware of only two studies indicating the sex ratios of hawksbill hatchlings produced on Caribbean nesting beaches, at Jumby Bay, Antigua (Mrosovsky et al., 1992) and Buck Island, U.S. Virgin Islands (Wibbels et al., 1999). In Antigua, the sand at nest depth was generally cooler than the measured pivotal temperature, suggesting a male bias in the hatchlings produced. In contrast, examination of the sex of hawksbill hatchlings that died from natural causes at Buck Island, suggested a strongly female-biased turtle output there. Bowen et al. (1996) indicated that a high proportion of the Mona Island immature aggregation had mtDNA haplotypes found primarily in hatchlings at Buck Island, with Bass (1999) calculating a contribution of 41.5% of this rookery to the Mona aggregation from an expanded data set. The same studies suggested that only a small contribution (4.14%) was made by Antigua to the Mona foraging aggregation. Any male bias in the Antigua hatchlings would consequently have little effect on the sex ratios of immature hawksbills at Mona Island.

With sex ratios in the surrounding immature foraging grounds so highly female biased and a strong female bias present in a major contributing rookery, the question remains what explains the balanced sex ratio of immatures at Mona Island. The observation that Mona Island beaches, in contrast to other rookeries, are used year-round by nesting hawksbills (CED, pers. obs.), raises the possibility that the additional males are produced locally during the low temperature winter months. Genetic analyses have yielded estimates that the Mona nesting females are represented in the local feeding ground immatures at levels of 12.7% (Bowen et al., 1996) and 41% (Diaz-Fernandez et al., 1999). A contribution as large as the latter figure, combined with a high overall proportion of males in the Mona hatchlings, would reconcile the genetic profile of the Mona foraging ground immature turtles with the observed non–female-biased sex ratio of the aggregation. We are currently conducting a sex ratio study of the hawksbill hatchlings produced on Mona Island, which should clarify whether local sourcing plays a significant role in determining the sex ratio of the Mona feeding ground immatures.

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